AD______________

CONTRACT NUMBER DAMD17-93-C-3004

TITLE: Preclinical Pharmacodynamic and Pharmacokinetic Studies of Investigational New Drugs

PRINCIPAL INVESTIGATOR: Patricia Noker, Ph.D.

CONTRACTING ORGANIZATION: Southern Research Institute
Birmingham, Alabama 35255-5305

REPORT DATE: November 1996

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	November 1996	Annual (2 Nov 95 - 1 Nov 96)

4. TITLE AND SUBTITLE Preclinical Pharmacodynamic and Pharmacokinetic Studies of Investigational New Drugs

5. FUNDING NUMBERS
DAMD17-93-C-3004

6. AUTHOR(S)
Patricia Noker, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Southern Research Institute
Birmingham, Alabama 35255-5305

8. PERFORMING ORGANIZATION REPORT NUMBER
SRI-LIF-96-661-7869

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick
Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

During the past year of the contract, a study was initiated to evaluate, in dogs, whether significant clinical or pharmacological interactions occur when single oral doses of the antimalarial drug WR238605 are given in combination with one of the following schizonticidal agents: mefloquine, halofantrine, chloroquine or quinine/doxycycline. An initial pilot experiment was conducted to obtain a profile of the plasma kinetics of each schizonticidal agent alone and in combination with WR238605 in order to establish optimal times for sample collection and physiological evaluations in a subsequent main experiment. No gross adverse physiological effects were observed when WR238605 was administered alone or in combination with the individual schizonticidal agents. Analyses of the plasma samples for drug concentrations have not been completed to date. Thus, conclusions relevant to possible kinetic interactions between WR238605 and the other schizonticidal agents cannot be drawn at this time.

14. SUBJECT TERMS Pharmacokinetics, Drugs, CD
WR238605, schizonticidal agents, drug interactions

15. NUMBER OF PAGES
18

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

FOREWARD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

__X__ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Patricia E. Noker 11/18/96
Patricia E. Noker, P.I. Date

Approved By:

Duane B. Lakings 18 Nov 96
Duane B. Lakings Date
Manager, Bioanalytical Chemistry

SRI-LIF-96-661-7869

Table of Contents

I. INTRODUCTION

The overall objective of this contract is to perform studies in rodents, dogs and/or monkeys on the pharmacokinetic and pharmacodynamic properties of drugs which are under development by the U.S. Army Medical Research and Development Command. Work under this contract is executed on a task order basis. The pharmacokinetic aspect of the investigations involves an assessment of the absorption, disposition, metabolism (biotransformation) and elimination of test compounds in experimental animals. The pharmacodynamic aspect involves relating certain measured parameters, for example, the production of methemoglobin, to blood and plasma levels of test compound and/or metabolites.

Information derived from these studies is intended to provide a data base for establishing an appropriate species and appropriate doses for subsequent subchronic and chronic toxicity studies, predicting possible organ toxicities which might occur and making critical decisions concerning the continued development of a drug. In addition, the studies generate data required by the Food and Drug Administration prior to submission of a Notice of Claimed Investigational Exemption for a New Drug (IND) and New Drug Applications, Human Use (NDA).

Previously on this contract, pharmacokinetic/pharmacodynamic and metabolism studies were conducted on three compounds which have shown potential as either anti-cyanotic agents or antidotes to anticholinesterase (nerve) agents. Specifically, the agents studied were:

•WR242511, an 8-aminoquinoline analogue of the widely used anti-malarial agent, primaquine, which was initially developed as a potential replacement for primaquine. The pharmacokinetics, pharmacodynamics, oral bioavailability and metabolism of WR 242511 were investigated in dogs (Task Order 93-02).

•HI-6 (WR249655), an H-oxime efficacious against soman-induced toxicity. The pharmacokinetics and metabolism of HI-6 in dogs were investigated (Task Order 93-03).

•PAHP (p-aminoheptanophenone, WR269410), an anti-cyanide agent. The pharmacokinetics, pharmacodynamics, oral bioavailability and metabolism of PAHP were studied in both dogs and rats (Task Orders 94-04 and 94-05).

In each of these studies, a radiolabeled formulation of the test compound was utilized. For each study, the basic approach was as follows:

a.) to determine the concentration of radioactivity in blood, red blood cells and plasma at various times after administration of the radiolabeled test compound.

b.) to determine the time-concentration profile of unchanged test compound and metabolites in plasma.

c.) to determine the rate and extent of urinary and fecal elimination of radiolabel.

d.) for the anticyanotic agents, to follow the time course of methemoglobin production.

e.) to isolate and identify the major metabolites of the test compound excreted in urine and feces.

Background information relevant to these compounds was provided in our 1994 and 1995 annual reports. During the past year of this contract, draft final reports on the pharmacokinetic and pharmacodynamic results obtained during the studies conducted with PAHP in dogs and in rats (Task Orders 94-04 and 94-05, respectively) were submitted to the COR. Since much of the data obtained during these two studies was reported previously in our 1994 annual report, only abstracts of these reports are provided herein (See Appendix A).

In addition, during the past year of the contract, a study was initiated to evaluate, in dogs, whether significant clinical or pharmacokinetic interactions occur when single doses of WR238605 are given in combination with either mefloquine, halofantrine, chloroquine or quinine/doxycycline (Task Order 95-06). A main goal of this study is to obtain safety data to support further drug development studies in man. The rationale for the study is provided below.

WR238605 is an antimalarial drug under clinical development that has many benefits over currently available drugs. It is more potent, less toxic and longer acting than primaquine, a related 8-aminoquinoline antimalarial drug. WR 238605 also has a much longer elimination half-life in man than does primaquine, making it a long-acting agent with the potential for a practical dosing regimen of once weekly administration. Its primary proposed use is as a prophylactic agent against *P. flaciparum*, with a secondary indication of treatment of *P. vivax* malaria.

In the event individuals on prophylactic WR238605 did develop malaria, treatment with an alternative blood schizonticidal agent would be required. Due to the long half-life of WR 238605, significant amounts of the drug would be present in the body when the blood schizonticidal agent was being administered. Similarly, in an individual with *P. vivax* malaria, he/she would likely be treated with a blood schizonticidal agent, followed by WR238605 for radical cure of the liver stage. Given the long half-lives of many schizonticidal agents, it is likely that significant amounts would still be present when WR238605 therapy was started. It is important, therefore, to know whether significant pharmacological interactions occur between WR238605 and other antimalarial agents.

The known toxicities in humans and clinical pharmacokinetic information available for the antimalarial agents WR 238605, mefloquine, chloroquine, halofantrine, quinine and doxycycline are provided below.

WR238605 is effective when administered orally and has potential toxic effects in the following systems: central nervous (headache), hematologic (hemolytic anemia, methemoglobinemia, thrombocytopenia), gastrointestinal (nausea, diarrhea), hepatic (LFT elevation) and pulmonary (alveolar proteinosis and inflammation). Peak blood concentrations are achieved at about 8-12 hr after oral administration. Its elimination half-life ranges from 1-2 weeks (1).

Mefloquine is effective when administered orally and has the following potential toxicities: gastrointestinal upset (nausea, vomiting, diarrhea, abdominal pain), central nervous system (headache, dizziness, vivid dreams), dermatologic (rash, pruritus). After oral administration, plasma concentrations of mefloquine increase biphasically and maximum concentrations are achieved about 17 hr after administration. The compound has a terminal half-life of about 20 days (2).

Chloroquine is a 4-aminoquinoline that is effective when administered orally, intravenously or intramuscularly. It has the following potential toxicities: gastrointestinal disturbances, pruritus, headache and visual disturbances. Chloroquine has complicated pharmacokinetics and different half-lives depending on the duration of dosing. Presumably this is due to multi-compartment characteristics becoming more apparent with increasing drug levels. Peak plasma concentrations are achieved 3 to 5 hr after oral administration. After single doses, the half-life of chloroquine increases from a few days to weeks as plasma concentrations decline; its subsequent terminal half-life is 30 to 60 days (2).

Halofantrine is orally administered and has the following potential toxicities: gastrointestinal (nausea, abdominal pain), cardiac (QT prolongation). Its absorption is increased with food, especially food high in fat (4-fold increase in concentrations). Peak blood concentrations occur at about 4 hours after oral administration. In man, halofantrine displays three distribution/elimination phases with half-lives of 2 hr, 1.5 days and 4 weeks (3).

Quinine is readily absorbed when given orally or intramuscularly and has the following potential toxicities: central nervous system (headache, tinnitus, visual disturbances), gastrointestinal (nausea, vomiting, abdominal pain, diarrhea), cardiac (QRS and QT prolongation, hypotension). Peak concentrations after oral administration occur at about 3 hr. In man, the terminal elimination half-life of quinine is 10-11 hr (2).

Doxycycline is an orally administered antimalarial used for prophylaxis and, when used in combination with quinine, for the treatment of malaria. Side effects which occur with doxycycline use include gastrointestinal disturbances, phototoxicity, hepatotoxicity and renal toxicity. Peak

plasma concentrations of doxycycline occur at about 2 hr after oral dosing. The elimination half-life of doxycycline is 16-18 hr (2).

The current drug interaction study with WR238605 consists of two phases: a.) an initial pilot experiment, involving one dog per dosing regime, to obtain a profile of the plasma kinetics of each schizonticidal agent alone and in combination with WR238605 in order to establish the optimal times of sample collection and physiological evaluations; b.) a main experiment, involving six dogs per dosing regime, to determine whether changes in plasma drug concentrations of WR238605 and the individual schizonticidal agents and/or various physiological parameters (as assessed by electrocardiographic measurements, methemoglobin production and hematological and clinical chemistry evaluations) occur following co-administration of these compounds.

II. Methods

A. Test Compounds

WR 238605 (8-[(4-amino-1-methylbutyl)amino]-2,6-dimethoxy-5-(3-trifluoromethylphenoxy) quinoline succinate), Bottle No. BM12562; chloroquine diphosphate, Bottle No. AU29291; mefloquine hydrochloride (WR 142490), Bottle No. BK11592; quinine sulfate hydrate, Bottle No. BM05870; doxycycline hydrochloride H_2O, Bottle No. AU29291; and halofantrine hydrochloride (WR 171669), Bottle No. BM01792 were supplied by Walter Reed Army Institute of Research (Washington, DC).

B. Dose Formulations

1. WR238605

A weighed quantity of WR238605 was added to a known volume of 1% carboxy methylcellulose (CMC)/0.5% Tween 80 to yield a formulation containing 10 mg WR238605/ml. The formulation was homogenized using a Teflon® probe until a uniformly dispersed (visual inspection) suspension was obtained.

2. Halofantrine

A weighed quantity of halofantrine was added to a known volume of 1% CMC/0.5% Tween 80 to yield a formulation containing 18 mg halofantrine/ml. The formulation was homogenized using a Teflon® probe until a uniformly dispersed (visual inspection) suspension was obtained.

3. Chloroquine

A weighed quantity of chloroquine was added to a known volume of 2% CMC/1% Tween 80 to yield a formulation containing 10 mg chloroquine/ml. The formulation was homogenized using a Teflon® probe until a uniformly dispersed (visual inspection) suspension was obtained.

4. Quinine

A weighed quantity of quinine was added to a known volume of 2% CMC/1% Tween 80 to yield a formulation containing 17 mg quinine/ml. The formulation was homogenized using a Teflon® probe until a uniformly dispersed (visual inspection) suspension was obtained.

5. Doxycycline

A weighed quantity of doxycycline was added to a known volume of 2% CMC/1% Tween 80 to yield a formulation containing 1.4 mg doxycycline/ml. The formulation was homogenized using a Teflon® probe until a uniformly dispersed (visual inspection) suspension was obtained.

6. Mefloquine

In the initial experiment, a weighed quantity of mefloquine was added to 1% CMC/0.5% Tween 80 to yield a formulation containing 18 mg mefloquine/ml. In addition, to circumvent emesis, an appropriate quantity of mefloquine was placed in a capsule to yield the desired dose/dog.

C. Dose Formulation Analysis

Upon preparation, a portion (0.2 to 0.5 ml) of the top, middle and bottom of each formulation was removed and stored below -15°C. Formulations were subsequently sent to Dr. Emil T. Lin at the University of California at San Francisco for analysis for drug concentrations.

D. Test Animals

Male Beagle dogs were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Dogs were quarantined for a minimum period of two weeks prior to use. During the quarantine period, each animal was given a complete physical examination which included hematological and clinical chemistry determinations. Body weights and rectal temperatures were monitored. Dogs were fed certified Purina dog chow (Purina Mills, Inc., St. Louis, MO) and given city tap water. The dogs were individually housed in stainless steel cages during both the quarantine and experimental periods. Each dog was identified by an ear tattoo.

All animal care and housing was conducted in accordance with the guidelines specified in the "Guide for Care and Use of Laboratory Animals" DHEW Publication No. NIH 86-23 (Revised, 1985), PL-99-198 of 1985, APHIS regulations, 9CFR, Parts 1, 2, 3 of 1989 and current AAALAC regulations.

E. Experimental Procedures

1. Dose Administration

During the past year of the contract, the pilot experiment was initiated. Dogs were fasted for approximately 16-20 hr prior to dosing. Dose formulation suspensions of WR238605 and each schizonticidal agent were administered by oral gavage. The doses administered were: 10 mg/kg of WR238605, 10 mg/kg of chloroquine, 18 mg/kg of halofantrine, 18 mg/kg of mefloquine, 17 mg/kg of quinine, and 1.4 mg/kg of doxycycline. These doses were scaled (by body weight) from the doses typically administered in clinical regimes.

Due to emesis which occurred after administration of oral suspensions of mefloquine, this compound was subsequently administered orally in a capsule.

Where WR238605 was given in combination with a schizonticidal agent, the schizonticidal agent was given at the following times after administration of WR238605: 0.25 hr (for mefloquine); 8 hr (for chloroquine); and 10 hr (for quinine and doxycycline).

2. Sample Collection

At each sample time, blood samples (3-4 ml) were collected from the jugular vein of individual dogs into a vacutainer containing heparin. For all dose groups, blood samples were collected at 0 min (prior to dosing) and at the following times after administration of the initial (or only) compound:

WR238605 only: 0.25, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72, 96, 120, 168, 336, 504 hr;
Mefloquine only: 0.25, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72, 96, 120, 168, 336, 504 hr;
Chloroquine only: 0.25, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72, 96, 120, 168, 336, 504 hr;
Halofantrine only: 0.25, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72, 96, 120, 168, 336, 504 hr;
Quinine + Doxycycline: 0.25, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72, 96, 120, 168 hr;
WR238605 + Mefloquine: 0.25, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72, 96, 120, 168, 336, 504 hr;
WR238605 + Halofantrine: 0.5, 2, 4, 8, 9, 10, 11, 12, 13, 16, 20, 24, 48, 96, 120, 168, 336, 504 hr;
WR238605 + Chloroquine: 0.5, 2, 4, 8, 9, 10, 11, 12, 13, 16, 20, 24, 48, 96, 120, 168, 336, 504 hr;
WR238605 + Quinine + Doxycycline: 0.5, 2, 4, 8, 10, 10.5, 11, 12, 13, 14, 20, 28, 48, 96, 120, 168, 336, 504 hr

3. Sample Analysis

Upon collection each blood sample was centrifuged to separate plasma. Plasma samples were stored at -70°C and subsequently shipped on dry ice to Dr. Emil T. Lin at the University of California at San Francisco (UCSF) for analysis for plasma drug concentrations.

III. RESULTS

With one exception, no adverse clinical signs or symptoms were observed following oral administration of WR 238605, halofantrine, chloroquine or quinine/doxycycline alone or after oral administration of WR 238605 in combination with these drugs.

For the dogs administered either mefloquine alone or mefloquine in combination with WR238605, emesis occurred within 30 min after administration of mefloquine when the drug was formulated in 1% CMC/0.5% Tween 80. In an attempt to circumvent emesis, a trial experiment was conducted in which mefloquine was prepared in various concentrations of CMC/Tween 80 and administered in different dose volumes. It was found that emesis still occurred when mefloquine was administered in a formulation containing lower concentrations of CMC/Tween 80 (0.2%/0.1%, respectively) or a ten-fold lower concentration of drug. Emesis did not occur, however, when vehicle only (either 1%CMC/0.5% Tween 80 or 0.5% CMC/0.25% Tween 80) was given. These results suggested that the emesis was drug related. Thereafter, dogs were administered mefloquine in a dissolvable capsule and emesis did not occur. Administration of mefloquine in a capsule was chosen, therefore, as the preferred method for oral administration of the drug.

To date, the results of the analyses of the plasma samples have not yet been received from Dr. Lin at UCSF.

IV. CONCLUSIONS

Since plasma drug concentration data are not yet available, no conclusions relevant to the effect of the schizonticidal agents on the plasma kinetics of WR238605 can be drawn. The initial clinical observations indicated that no gross physiological incidents occurred when WR238605 was given alone or in combination with either chloroquine, halofantrine, mefloquine or quinine/doxycycline. Data obtained during the main study, which will be initiated within the next several months, should provide information which will indicate whether co-administration of WR238605 with these antimalarial agents poses a potential health threat.

V. REFERENCES

1. R. Brueckner, personal communication.

2. J.M. Tracey and L.T. Webster, Jr., in *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, J.G. Hardman and L.E. Limbird, Eds. (The McGraw-Hill Companies, Inc., New York, NY, 1996).

3. J. Karbwang and K.N. Bangchang, Clin. Pharmacokinet.**27**, 104 (1994).

APPENDIX A

Abstracts of the Study Reports for:

p-Aminoheptanophenone (PAHP) (WR 269410) Single Dose IV and Oral Pharmacokinetic, Pharmacodynamic, Bioavailability and Metabolism Study in Dogs: Part I: Bioavailability and Pharmacokinetics (Task Order 94-04)

and

p-Aminoheptanophenone (PAHP) (WR 269410) Single Dose IV and Oral Pharmacokinetic, Pharmacodynamic, Bioavailability and Metabolism Study in Rats: Part I: Bioavailability and Pharmacokinetics (Task Order 94-05)

p-Aminoheptanophenone (PAHP) (WR 269410) Single Dose IV and Oral Pharmacokinetic, Pharmacodynamic, Bioavailability and Metabolism Study in Dogs: Part I: Bioavailability and Pharmacokinetics

PAHP (para-aminoheptanophenone) is currently under development as an anti-cyanotic agent. The disposition, metabolism, bioavailability and pharmacodynamics (as assessed by methemoglobin production) of [^{14}C]PAHP were investigated in a cross-over study in which five male beagle dogs were administered an oral and IV dose of 7 mg/kg. Following oral administration of [^{14}C]PAHP, peak blood levels of radioactivity were achieved in dogs within 45 to 90 min; thereafter, the dogs eliminated radioactivity from whole blood in two apparent phases with mean half-lives of 1.5 hr and 31.8 hr. Similar disposition half-lives were observed after administration of the same dose IV. Following either route of administration, the levels of radioactivity in red blood cells increased with time after dosing, relative to the levels in whole blood and plasma, such that beyond 12 hr post dose most of the radioactivity in blood was associated with the red blood cells. Mean half-lives computed for the apparent two phase elimination of radioactivity from red blood cells were 0.9 hr and 74.0 hr after oral administration of [^{14}C]PAHP, and 2.0 hr and 65.0 hr after IV administration. In plasma, radioactivity was also eliminated in two apparent phases with mean half-lives of 1.2 hr and 34.7 hr following oral dosing, and 2.6 hr and 33.7 hr following IV dosing. Highest concentrations of unchanged PAHP, as determined by reversed phase HPLC, were observed in plasma immediately after administration of the IV dose, and at 30 to 90 min after the oral dose. Unchanged PAHP was subsequently eliminated in two apparent phases with mean half-lives of 1.0 hr and 15.6 hr following oral administration, and 2.0 hr and 19.3 hr after IV administration. The mean oral bioavailability of radioactivity derived from [^{14}C]PAHP was estimated to be 63.0%. For unchanged PAHP, the mean oral bioavailability in plasma was estimated to be 50.9%. Peak concentrations of methemoglobin in whole blood were achieved at 2 to 3 hr after either an oral or IV dose of [^{14}C]PAHP. Following either route of administration of [^{14}C]PAHP, a direct relationship was not observed between methemoglobin production and either the levels of radioactivity or unchanged PAHP in blood and plasma. After oral administration of [^{14}C]PAHP, the mean urinary elimination of radioactivity by the dogs accounted for 59.2% of the dose and the fecal elimination, 27.3%. With IV dosing, mean values of 71.5% and 21.8% of the dose were eliminated in urine and feces, respectively. The results of HPLC analyses indicated that no unchanged [^{14}C]PAHP was eliminated in urine following either oral or IV administration of the compound; most of the radioactivity in urine was associated with three major radiolabeled metabolites. Unchanged PAHP was detected in feces following both oral and IV administration of [^{14}C]PAHP. In addition, two major radiolabeled metabolites, and several minor metabolites, were found in feces. Studies currently are underway to identify the major urinary and fecal metabolites of PAHP which are produced by dogs.

p-Aminoheptanophenone (PAHP) (WR 269410) Single Dose IV and Oral Pharmacokinetic, Pharmacodynamic, Bioavailability and Metabolism Study in Rats: Part I: Bioavailability and Pharmacokinetics

PAHP (para-aminoheptanophenone) is currently under development by the U.S. Army as an anti-cyanotic agent. The disposition, metabolism, bioavailability and pharmacodynamics (as assessed by methemoglobin production) of [U-^{14}C]PAHP has been investigated in rats administered both an oral and IV dose of 7 mg/kg. Following oral administration of [^{14}C]PAHP to rats, peak levels of radioactivity were achieved in whole blood within 30 min to 45 min after dosing. Beyond this time, rats eliminated radioactivity from whole blood in an initial phase of 1.3 hr and then in a prolonged phase of 39.0 hr. The corresponding disposition half-lives after IV administration of the same dose were 1.1 hr and 46.8 hr. The mean oral bioavailability of radioactivity derived from [^{14}C]PAHP was estimated to be 74.2%. Following both oral and IV dosing, the levels of radioactivity associated with red blood cells increased with time, relative to the levels in whole blood and plasma, suggesting that [^{14}C]PAHP and/or its radiolabeled metabolite(s) were being sequestered in red blood cells. Mean half-lives for the terminal disposition of radioactivity from red blood cells were 60.1 hr and 63.5 hr after oral and IV dosing, respectively. Following either route of dose administration, the half-lives for the concurrent disposition of radioactivity from plasma were approximately three to four times shorter than the half-lives in red blood cells. Highest concentrations of unchanged PAHP, as determined by reverse phase HPLC, were observed in plasma within 15 min to 45 min after oral administration of [^{14}C]PAHP and at the earliest time point (15 min) after IV dosing. After oral dosing, plasma concentrations of unchanged PAHP were 7 to 12 times lower than the corresponding plasma concentrations of radioactivity; after IV dosing, the concentrations of unchanged PAHP in plasma were 6 to 7 times lower than plasma radioactivity levels. The terminal disposition half-life for unchanged PAHP was 3.7 hr after oral dosing and 2.7 hr after IV dosing. For unchanged PAHP, the mean oral bioavailability in plasma was estimated to be 54.9%. Mean peak concentrations of methemoglobin were observed in whole blood at 45 min after either oral or IV administration of [^{14}C]PAHP. Following either route of administration of [^{14}C]PAHP, no direct correlation was found between methemoglobin production and the levels of radioactivity in whole blood, plasma and red blood cells, and plasma concentrations of unchanged PAHP. At 12 hr after either oral or IV administration of [^{14}C]PAHP, highest levels of radioactivity were found in the liver and small and large intestines; significant levels of radioactivity were not detected in either brain or muscle. After oral administration of [^{14}C]PAHP, the mean urinary elimination of radioactivity during the first 48 hr after dosing accounted for 71.2% of the dose and the fecal elimination, 22.1%. For rats given an IV dose, a mean value of 79.3% of the dose was eliminated in urine and 13.7% in feces from 0 to 48 hr after dosing. At 48 hr, the percentage of the dose remaining in the carcass was the same after oral and IV dosing and accounted for a mean value of 1.2%. The results of reverse phase HPLC analyses indicated that the metabolite profiles of urine were similar after either oral or IV administration of [^{14}C]PAHP. Unchanged PAHP was not detectable in urine collected at any time after either route of administration of [^{14}C]PAHP; most of the radioactivity in urine was associated with two major

metabolite peaks. Unchanged PAHP and two major radiolabeled metabolites, which were different from those in urine, were detected in feces after either oral or IV administration of [^{14}C]PAHP. Studies are in progress to identify the major urinary metabolites of PAHP.